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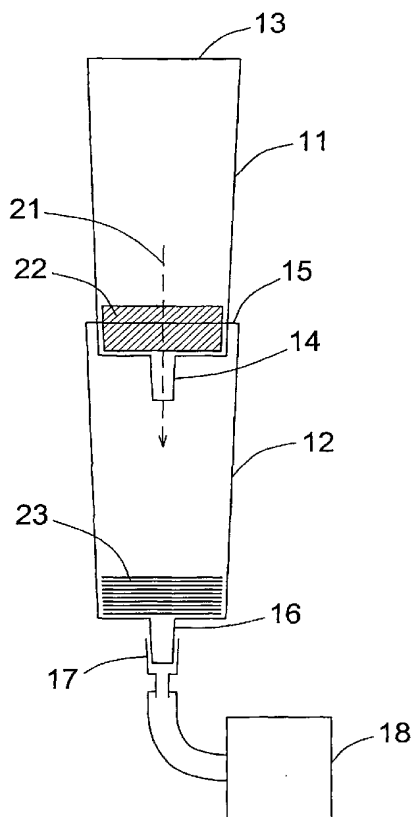
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[Continued on next page]

(54) Title: FLOW-THROUGH SYSTEM FOR LYSATE CLARIFICATION AND NUCLEIC ACID BINDING IN A SINGLE STEP



(57) Abstract: Two flow-through receptacles, one containing a biologic lysate filtration medium and the other a nucleic acid binding medium, are constructed to be releasably joinable to form a continuous leak-free flow-through passage with the filtration medium at an upstream location in the passage and the binding medium at a downstream location. The joined receptacles are attached to a vacuum source arranged to draw a vacuum through the lower (nucleic acid binding) receptacle, or placed in a centrifuge, and a biologic lysate is placed in the upper (filtration) receptacle. With a single application of vacuum or centrifugation, the lysate is clarified and nucleic acids are extracted simultaneously. The receptacles are then separated and the purified and extracted nucleic acids are recovered from the nucleic acid binding receptacle.



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# **FLOW-THROUGH SYSTEM FOR LYSATE CLARIFICATION AND NUCLEIC ACID BINDING IN A SINGLE STEP**

## **BACKGROUND OF THE INVENTION**

In research facilities, diagnostic clinics, forensic laboratories, and other environments where biological systems are studied, biologists are often called upon to identify a gene, determine a nucleic acid sequence, learn the function of a particular protein, or compare a nucleic acid sequence from one source with that from another. Among the first steps in these endeavors is the lysing (disruption) of biological materials (such as mammalian cells, bacteria, yeast, tissues, etc.) by a variety of established methods, both physical and chemical. The crude lysate is typically clarified either by filtration or centrifugation to remove cellular debris and other contaminants. The nucleic acid, which may be genomic/chromosomal DNA, extrachromosomal DNA (such as plasmid DNA), or RNA, is then extracted or segregated from the clarified lysate, typically by attachment to a solid support or by some other selective capture method. The nucleic acid and the substrate to which it is attached are then washed to remove residual biological debris, salts and other reagents, and then detached and collected in its purified form.

The entire procedure requires several steps and is often incompatible with the throughput requirements of various research or diagnostics facilities. It is therefore highly desirable to consolidate or eliminate as many steps or handling requirements as possible.

## **SUMMARY OF THE INVENTION**

The present invention resides in an apparatus that allows one to perform the clarification of a biological cell lysate and the extraction of nucleic acids from the clarified lysate in a single step. This is achieved with an apparatus which includes two flow-through receptacles that are joined to form a single flow-through passage in which lysate clarification and nucleic acid binding are performed at an upstream and a downstream location, respectively. The apparatus is constructed to allow the receptacles, once joined, to be separated so that the nucleic acid which has become bound to the

binding medium in the second receptacle can be washed and then recovered in a purified state in an elution buffer. The two receptacles are arranged such that flow through the passage can be imposed by the application of a vacuum (which term is used here to include a partial vacuum), centrifugation, or by other external means, and the receptacles are joined by any of a variety of methods that will seal the passage and allow a negative pressure to be established throughout the system, for the purpose of minimizing, and preferably preventing entirely, the leakage of fluid or gas either into or out of the passage at the joiner of the receptacles. The seal is releasable however when the receptacles are disengaged.

The term "receptacle" is used herein to encompass both single-vessel receptacles such as tubes or columns, and multi-vessel receptacles such as microtiter plates or any other plates or trays that contain an array of wells or orifices. As will be seen from the discussion that follows, the most practical releasable sealing means will differ depending on whether a single-vessel or a multi-vessel receptacle is used, and whether vacuum or centrifugation is employed.

### BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a front elevation view of one example of an apparatus in accordance with the present invention in which each receptacle consists of a single column.

FIG. 2 is a front elevation view of a second example of an apparatus in accordance with the present invention in which each receptacle consists of a single column.

FIG. 3a is a front elevation view of an example of an apparatus in accordance with the present invention in which each receptacle consists of a multi-well plate, the plates shown in position prior to being joined for use in accordance with this invention.

FIG. 3b is a similar view of the apparatus of FIG. 3a in which the plates are joined for the first stage of the procedure, i.e., lysate filtration and nucleic acid binding.

FIG. 3c is a similar view of the apparatus of FIG. 3a in which the plates are separated for the second stage of the procedure, i.e., the washing step.

FIG. 4a is a front elevation view of a variation of the apparatus of FIGS. 3a, 3b, and 3c, with the two plates separated.

FIG. 4b is a similar view of the apparatus of FIG. 4a with the plates joined.

FIG. 5a is a front elevation view of a further variation of the apparatus of FIGS. 3a, 3b, and 3c, with the two plates separated.

FIG. 5b is a similar view of the apparatus of FIG. 5a with the plates joined.

### **DETAILED DESCRIPTION OF THE INVENTION AND PREFERRED EMBODIMENTS**

While this invention is susceptible to a wide range of configurations, arrangements and embodiments, the following discussion will focus on certain specific examples, the structural and functional aspects of which will serve to provide an understanding of the invention as a whole.

FIG. 1 depicts an apparatus formed from two single tubes or columns, one 11 serving as a filtration (or clarification) column and the other 12 as a nucleic acid binding column. Both columns have open inlet and outlet ends. The filtration column 11 is arranged with its inlet end 13 at the top and its outlet end 14 at the bottom. The binding column is likewise arranged with its inlet end 15 at the top and its outlet end 16 at the bottom. The binding column 12 terminates at its outlet end 16 in a male "Luer"-type connector that mates with a corresponding female "Luer"-type connector 17 on a vacuum manifold 18 or on any port that is connected to a vacuum source. The "Luer"-type connectors referred to in this description are merely one example of complementary connectors in general, a variety of which are known and any of which can be used. Alternatively, the two columns can be placed in a centrifuge tube and a centrifuge substituted for the vacuum manifold, provided that the end 16 of the nucleic acid binding column is maintained above the bottom of the centrifuge tube.

The two columns are arranged vertically with the filtration column 11 above the binding column 12, and the dimensions and contours of the two columns are such that the lower portion of the filtration column 11 can be inserted through the inlet end 15 of the binding column. The contacting surfaces of the two columns are complementary in shape and the two columns form a friction fit that holds them together and prevents air leakage around the lower end of the filtration column into the interior of

the columns. The two columns thus form a single continuous flow passage in the direction of the dashed-line arrow 21.

The lower end of the filtration column 11 is shaped to retain a solid filtration medium 22, which may be in the form of a cake, a packed bed of nonadherent particles, a porous membrane, a stack of porous membranes, or any other configuration that will function as a filter. The lower end of the binding column 12 is likewise shaped to retain a solid material 23 that binds nucleic acids. This material as well may be in the form of a cake, a packed bed of nonadherent particles, a porous membrane, a stack of porous membranes, or any other material with the appropriate binding affinity for nucleic acids.

To use the apparatus of FIG. 1, the clinician simply inserts the filtration column 11 into the binding column 12 as shown, both columns already containing their respective solid media, and presses the two together with sufficient force to form a tight fit. The male Luer connection (or other terminus) at the outlet end 16 is then joined to the female Luer connection 17 (or other complementary fitting) of the vacuum apparatus or manifold 18. The crude lysate is then placed in the filtration column through the open inlet end 13 at the top of the column and vacuum is applied until all liquid from the lysate has passed out of the both columns. The vacuum is then turned off, the filtration column 11 is removed from the binding column 12, and a wash buffer is placed in the binding column through its now exposed upper end 15. Following the wash step, an elution buffer is introduced and the binding column is transferred to a centrifuge tube, where the purified nucleic acid is detached from the binding column and collected via centrifugation. Alternatively, elution can be vacuum-mediated with deposition of the product into an appropriate receptacle.

The apparatus of FIG. 2 is similar to that of FIG. 1 except that an adapter cap 25 is used between the two columns to form the air-tight friction fit. The adapter cap 25 has an opening 26 that receives the downwardly extending tip 27 at the lower end of the filtration column in a close fit. Another close fit, preferably a friction fit, exists between the outer surface 28 of the adapter cap and the opening at the top of the binding column 12. These close fits together seal the internal passageway enough to hold a vacuum. The procedure for use of this apparatus can be the same as that described above in connection with FIG. 1.

FIGS. 3a, 3b, and 3c illustrate the features of the invention as they might be applied to multi-well plates. The three figures depict successive stages of the

apparatus before and during use. In FIG. 3a, the filtration receptacle is shown as a filtration plate 31 with an array of wells (only one of which 32 is visible) and the binding receptacle is shown as a binding plate 33, also with an array of wells (only one of which 34 is visible). The arrays of wells on the two plates are identical with each well in the filtration plate being in registration (i.e., alignment) with a well in the binding plate. A gasket 35 is placed between the two plates to form an air-tight seal to hold a vacuum. The gasket may extend only around the periphery of the wells or it may surround each individual well, in which case the gasket will contain an array of apertures with one aperture in alignment with each pair of aligned wells. Each well 32 in the filtration plate has a lower end shaped to retain the filtration medium 36, and the lower end of each well 34 in the binding plate is similarly shaped to retain the binding medium 37.

In FIG. 3b, the two plates 31, 33 and gasket 35 are placed in contact with the appropriate alignment. Once combined in this manner, the plates and gasket are placed on a vacuum manifold that draws a vacuum downward through the bottom of the binding plate. Lysates are placed in the wells of the upper plate 31, vacuum is applied, and the liquid components of the lysates are allowed to pass through the plates in the direction of the arrows 41, 42, 43 toward the vacuum source. Alternatively, the joined plates can be placed in a centrifuge apparatus with the appropriate rotor and stages. Either way, the lysates will be clarified as they pass through the filtration plate 31, and the nucleic acid will immediately be extracted from the clarified lysates by binding of the nucleic acid in the binding plate 33.

The gasket 35 may be coated with adhesive to assist in the avoidance of leaks and cross contamination. Preferably, adhesive is applied in such a manner as to cause the gasket to preferentially adhere to the upper plate so that when the two plates are separated from each other, the gasket will remain adhered to the upper plate leaving the lowering plate fully accessible and gasket-free for further introduction of liquids such as the wash buffer and the elution buffer. This can be achieved by limiting the adhesive to the upper surface 44 of the gasket (the surface in contact with the filtration plate 31) or by using two different adhesives, one on the upper surface 44 and another on the lower surface 45 (the surface in contact with the binding plate), the upper adhesive being the stronger of the two so that the gasket is less easily separated from the upper plate than the lower. The selection or formulation of adhesives that will accomplish this result is a matter well within the expertise of adhesives suppliers and many such materials are well

known and readily available. In certain applications, the adhesive can be eliminated entirely while still achieving an acceptable result.

After the vacuum or centrifuge has drawn all liquid from the stacked plates, the two plates are separated as shown in FIG. 3c. Since the adhesive on the upper surface 44 of the gasket 35 forms a stronger bond than the adhesive on the lower surface 45, the gasket remains adhered to the filtration plate 31, leaving the binding plate 33 free of the gasket and ready for further processing. This processing will typically include the addition of a wash buffer into the wells 34 of the binding plate through the now exposed top openings of the wells, and the reapplication of the vacuum (or centrifugation) to draw the wash buffer through the binding medium thereby removing any unbound material and reagents from the binding medium. Finally, an elution buffer is placed in the wells 34 of the binding plate, and vacuum (or centrifugation) is applied once again to remove the nucleic acids from the binding medium. The eluate containing the purified nucleic acid can be collected in a 96-well plate, in microtubes, or in any other vessels, and is ready for analysis including use of the polymerase chain reaction if desired.

FIGS. 4a and 4b illustrate a variation on the multi-well plate configuration of FIGS. 3a, 3b, and 3c. In this variation, the filtration plate 51 has wells 52 with lower extensions 53 that fit inside the wells 54 of the nucleic acid binding plate 55 in a friction fit to form an air-tight seal or a seal of sufficient strength to hold the two plates together when a centrifugal force is applied. FIG. 4a shows the plates separated, while FIG. 4b shows them combined.

FIGS. 5a and 5b illustrate a further variation. In this variation, a contoured plate adapter 61 is placed between the filtration plate 62 and the nucleic acid binding plate 63. The adapter functions in a manner analogous to the adapter cap 25 of FIG. 2. FIG. 5a shows the two plates separated with the adapter 61 attached to the nucleic acid binding plate 63 by a friction fit, while FIG. 5b shows the plates and adapter combined.

Lysates intended for purification with the apparatus of the present invention can be prepared by conventional methods. A wide variety of lysis methods are known to those skilled in the art, and the choice of the appropriate method for any particular case will depend on the type of nucleic acid to be extracted and purified, the type of biological material from which the nucleic acid is to be extracted and the condition of the biological material at the time of lysis, and the procedures that are to be performed on the nucleic acid once purified. The various possibilities as well as the



knowledge of which are the most appropriate in any particular case are well known to those skilled in the art.

Filtration and binding media that are known to be effective in lysate clarification and nucleic acid binding, respectively, can be used in the procedures described above. The various choices of wash buffers and elution buffers will likewise be readily apparent to those skilled in the art. For purifying plasmid DNA, for example, examples of effective filtration medium are porous polyethylene membranes, polymeric mesh, and filter paper. Examples of an effective binding medium are borosilicate membranes, an example of an effective wash buffer is buffered ethanol solution, and an example of an effective elution buffer is buffered, low-salt solution. In embodiments involving the use of a gasket with two adhesives, adhesives of a wide range of adhesion strengths are commercially available.

The foregoing is offered primarily for purposes of illustration. Further alternatives as well as modifications and variations of the configurations, systems, materials, and procedural steps described above, which will be apparent to those skilled in the art upon reading this specification, are included within the scope of this invention.

**WE CLAIM:**

1. Apparatus for single-step clarification of a biological lysate and recovery of nucleic acids therefrom, said apparatus comprising:
  - a filtration medium capable of retaining solid biological contaminants and thereby clarifying said lysate, said filtration medium retained in a first flow-through receptacle;
  - a binding medium capable of binding nucleic acids, said binding medium retained in a second flow-through receptacle; and
  - means for releasably joining said first and second flow-through receptacles in substantially air-tight manner to form a single fluid flow path passing first through said first flow-through receptacle and then through said second flow-through receptacle.
2. Apparatus in accordance with claim 1 further comprising vacuum means for imposing vacuum-induced flow through said second flow-through receptacle.
3. Apparatus in accordance with claim 1 in which said first and second flow-through receptacles are single tubes defined as first and second tubes, respectively, each such tube having an inlet end and an outlet end.
4. Apparatus in accordance with claim 3 in which said means for releasably joining said first and second flow-through receptacles are comprised of complementary shapes of said outlet end of said first tube and said inlet end of said second tube, such that substantially air-tight contact is created by insertion of said outlet end of said first tube into said inlet end of said second tube.
5. Apparatus in accordance with claim 3 in which said means for releasably joining said first and second flow-through receptacles are comprised of adapter means to receive said outlet end of said first tube and said inlet end of said second tube to form a substantially air-tight connection between said first and second tubes.
6. Apparatus in accordance with claim 1 in which said first and second flow-through receptacles are multi-well plates defined as first and second plates, each such plate comprising an array of flow-through wells.

7. Apparatus in accordance with claim 1 in which said means for releasably joining said first and second flow-through receptacles are comprised of a gasket shaped to fit between said first and second plates and to form a substantially air-tight seal around each well of each plate.

8. Apparatus in accordance with claim 7 in which said means for releasably joining said first and second flow-through receptacles further comprises an adhesive on said gasket causing said gasket to preferentially adhere to said first plate

9. Apparatus in accordance with claim 7 in which said means for releasably joining said first and second flow-through receptacles further comprises a first adhesive on one side of said gasket and a second adhesive on an opposing side of said gasket, said first adhesive forming a stronger bond than said second adhesive such that when said gasket is adhered to both said first and second plates by said first and second adhesives, forcible disengagement of said first and second plates from each other causes said gasket to remain adhered to said first plate.

10. Apparatus in accordance with claim 1 in which said filtration medium is comprised of a porous membrane.

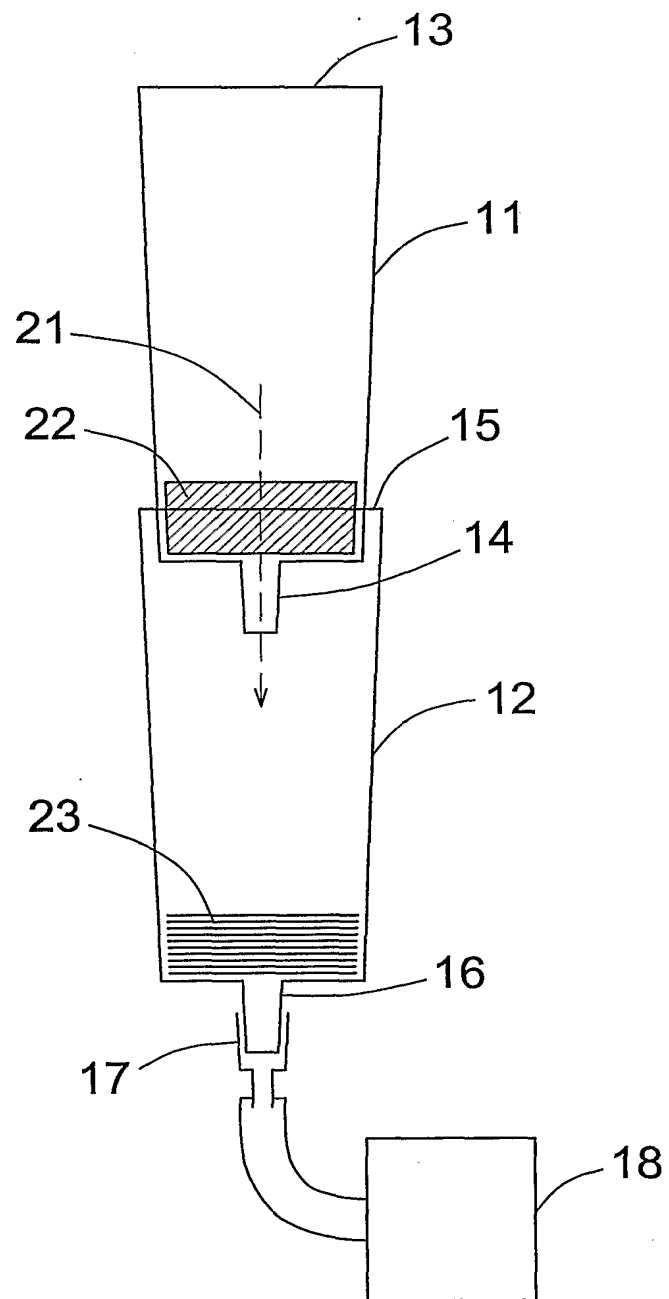
11. Apparatus in accordance with claim 1 in which said filtration medium is comprised of a plurality of porous membranes.

12. Apparatus in accordance with claim 1 in which said binding medium is comprised of a porous membrane.

13. Apparatus in accordance with claim 1 in which said binding medium is comprised of a plurality of porous membranes.

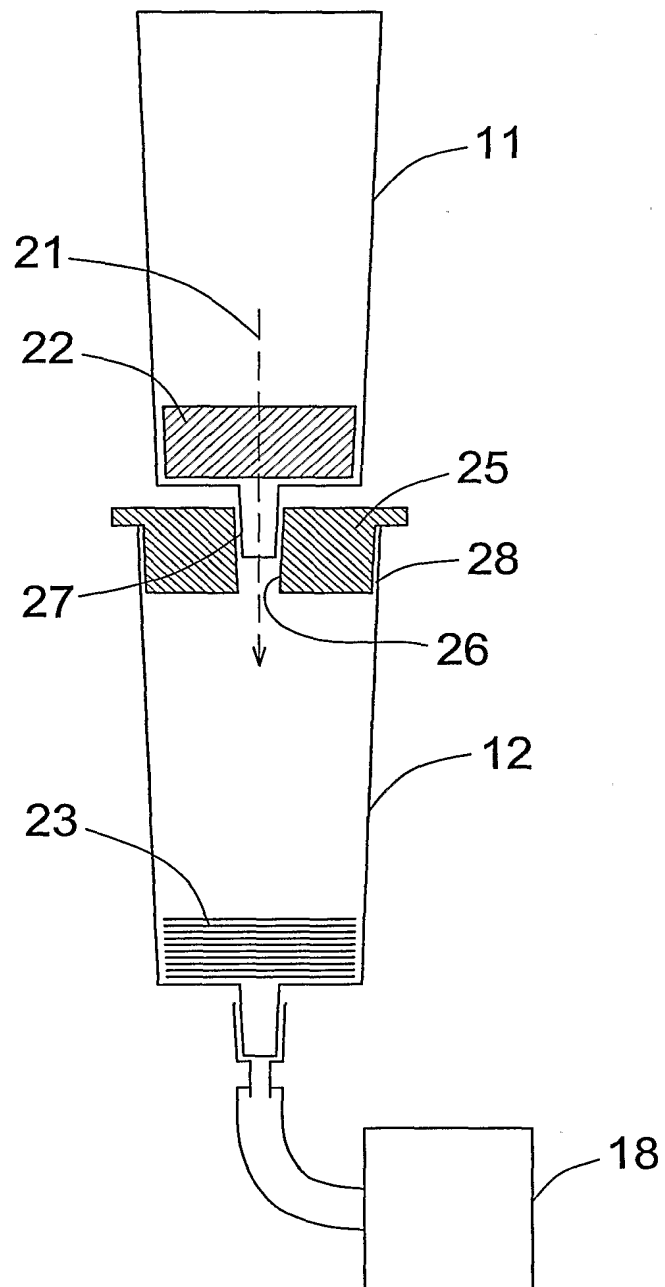
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Fig. 1



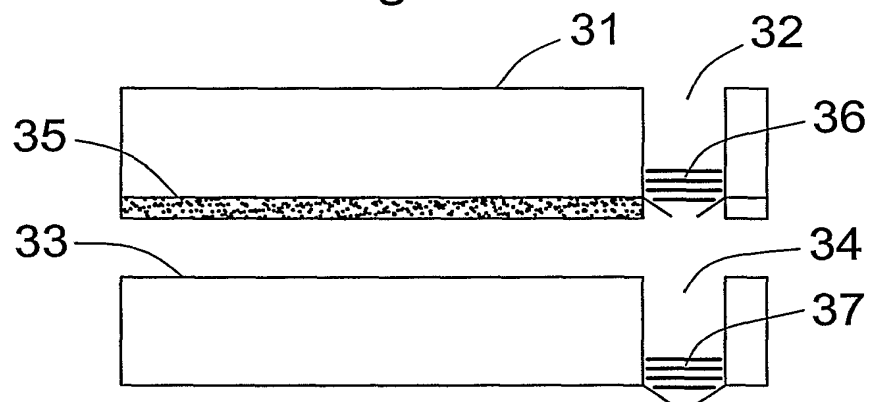
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Fig. 2

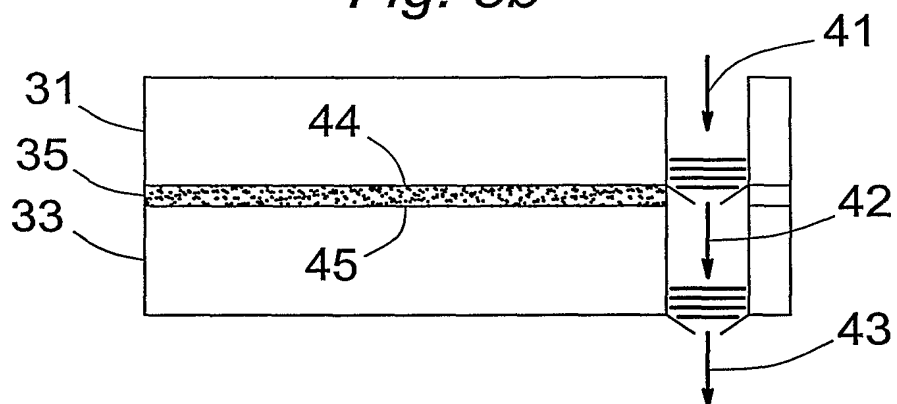


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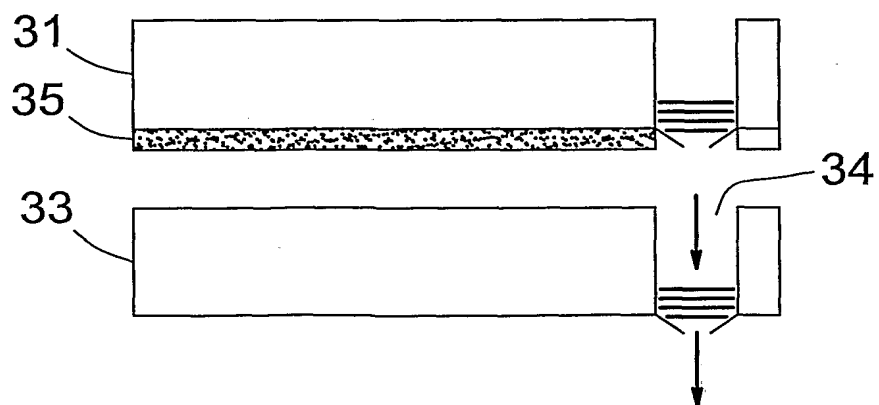
*Fig. 3a*



*Fig. 3b*

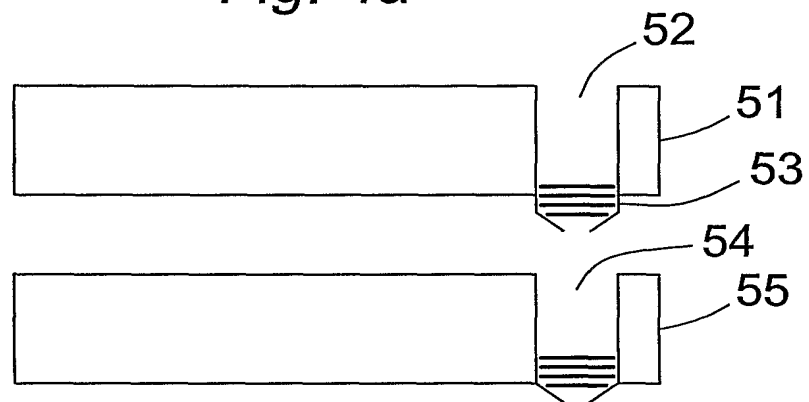


*Fig. 3c*

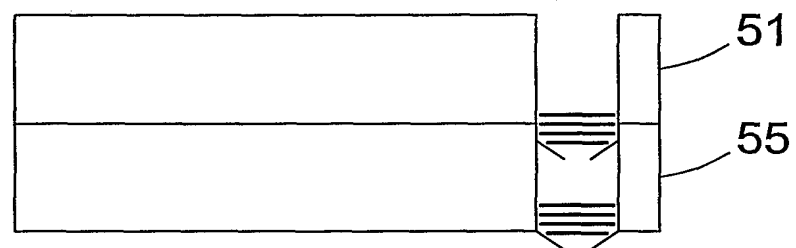


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*Fig. 4a*

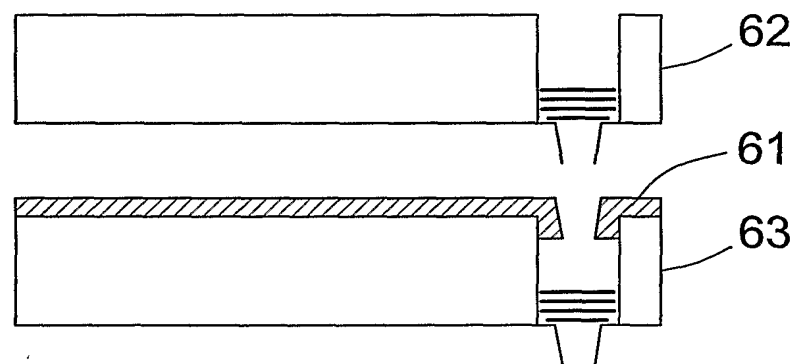


*Fig. 4b*



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*Fig. 5a*



*Fig. 5b*

